

Research Article

A New Method for Fabrication of Nanohydroxyapatite and TCP from the Sea Snail *Cerithium vulgatum*

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Biphasic bioceramic nanopowders of hydroxyapatite (HA) and β -tricalcium phosphate (TCP) were prepared from shells of the sea snail *Cerithium vulgatum* (Bruguière, 1792) using a novel chemical method. Calcination of the powders produced was carried out at varying temperatures, specifically at 400°C and 800°C, in air for 4 hours. When compared to the conventional hydrothermal transformation method, this chemical method is very simple, economic, due to the fact that it needs inexpensive and safe equipment, because the transformation of the aragonite and calcite of the shells into the calcium phosphate phases takes place at 80°C under the atmospheric pressure. The powders produced were determined using infrared spectroscopy (FT-IR), X-ray diffraction, and scanning electron microscopy (SEM). The features of the powders produced along with the fact of their biological origin qualify these powders for further consideration and experimentation to fabricate nanoceramic biomaterials.

1. Introduction

To date, biomaterials is a rapidly developing interdisciplinary field at the interface of engineering, science, and healthcare industry; its effect on human health related issues is also obvious and recognized all over the world. The global biomaterials device market was estimated as \$115.4 billion in 2008 and is expected to increase to \$252.7 billion in 2014. The largest market share among all biomaterial products belongs to orthopedic biomaterials [1], like hydroxyapatite (HA) materials. With a chemical formula of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, HA is the main inorganic component of bone [2] and tooth [3]. Thus, HA is very popular for implant materials

especially in orthopedic bone surgery [2] and other hard tissue implantations [3], such as in dental and aesthetic surgery.

Powders of HA can be produced with very various chemical techniques, such as precipitation, hydrothermal techniques, hydrolysis of other calcium phosphates, and sol-gel [4] from very pure chemicals or from natural materials. Calcination is another method to fabricate HA from different natural sources, like bone (i.e., human [5], bovine [6], sheep [7], turkey, and chicken) or tooth dentine [8] and enamel [9–11]. In previous work, there are also papers reporting some very interesting sources for HA production, such as crocodile bone [12], deer antler [13], and fish wastes.



FIGURE 1: Typical photos of shells of *Cerithium vulgatum* Bruguière, 1792, Mediterranean [30].



FIGURE 2: Habitation areas for *Cerithium vulgatum* [31].

Hydrothermal methods are very popular to transform various sources with a sea origin, such as cuttlefish bone [14], some oysters [15], and corals [16]. In our more recent studies, we have presented some very simple mechanochemical methods, which can be conducted with a simple hot-plate stirrer and with ultrasonic equipment [17, 18]. Various aragonitic structures, such as cuttlefish bone [19], sea [20] and land snail shells [21], sea urchin shells [17, 18, 20], various mussel shells [19, 22–24], pearl powder [25], corals [16], and calcite from egg shells [26], were successfully transformed into various Ca-phosphate bioceramic powders using these novel mechanochemical methods.

In this work, a novel and simple chemical method was utilised to fabricate nanobiphasic powders of HA and TCP from the shells of a local sea snail, *Cerithium vulgatum* Bruguière, 1792 [27]. The *Cerithium vulgatum* is a species of sea snail, which is a marine gastropod mollusk and also belongs to the family Cerithiidae [28]. Generally the *Cerithium vulgatum* shells are occupied by hermit crabs [29]. The typical shape of these shells is revealed in Figure 1 [30].

2. Materials and Experimental Procedure

Generally, the habitation areas for *Cerithium vulgatum* are all coastal areas of the United Kingdom, Spain, Portugal, Greece, and West of Turkey (Figure 2, [31]). These species can be obtained from the Black Sea in Turkey [32]. Thus, it is not surprising to come across with the empty shells of the sea snail *Cerithium vulgatum* Bruguière by the Marmara Sea, in Turkey, and specifically by the beaches of Princes Islands. However, the collection of the *Cerithium vulgatum* shells is generally difficult by the beaches of Istanbul since their number are quite small and are negligible in comparison to those of other collected shells. The other shells mainly

belong to the species *Nassarius hinniea reticulatus* (this species is overnumbered in these beaches). However, the shells of *Cerithium vulgatum* are easily recognized and separated from the shells of *Nassarius hinniea reticulatus* because the latter ones are much smaller in length and diameter than the former ones and have a brownish color.

Empty shells of a local sea snail (*Cerithium vulgatum* Bruguière, 1792) were taken from a local beach in Princes Islands, Heybeli Island (local beach name; German Beach) in Istanbul, Turkey. No living creatures were used in this study at all. The empty shells were cleaned thoroughly from sand particles and other foreign materials. Then, the shells were dried and crushed into small particles and finally planetary-milled in a porcelain jar. The milled powder was sieved using a 100 μm sieve (i.e., the particle size was $<100 \mu\text{m}$).

A small sample of the fine powder was analyzed using differential thermal and gravimetric analysis (DTA/TGA) to determine the exact CaCO_3 content. Batches of 2 g of powder were suspended in an aqueous solution of distilled water in a conical flask. Then, according to a previous study [33], solution of H_3PO_4 was added in such an amount as to satisfy the stoichiometric molar ratio of Ca/P equal to 1.667 (that corresponds to HA; this sample is hereafter designated as A) or 1.5 (that corresponds to TCP; this sample is hereafter designated as B). Hot-plate stirrer equipment was used with a conical flask in this work. The temperature of the solution was set at 80°C and the reaction took place for 8 h under continuous stirring. After that, the powders were removed from the liquid by filtration and dried at 100°C overnight in an incubator. The dried powders were calcined using an electric furnace (Nabertherm HT 16/17, Lilienthal, Germany) for 4 h in air. The powders of the sample A (i.e., Ca/P = 1.667) and the powders of the sample B (i.e., Ca/P = 1.5) were calcined at 800°C and 400°C , respectively.

To characterize the materials, in either the raw form or the final powders, the following equipment was used. The thermal analysis was determined using DSC-DTA-TG equipment (TA SDT Q600 Protherm). The observation of the microstructure of the samples was observed in a scanning electron microscope (SEM, JEOL JSM 7000F Field Emission Scanning Electron Microscope, equipped with a Hitachi 1000 Tabletop microscope). The crystalline phases developed in the calcined powders were used by X-ray diffraction analysis (Bruker D8 Advance X-ray diffractometer). The Fourier transform infrared (FT-IR) spectra of the produced powders were analyzed in a Bruker ALPHA FT-IR spectrometer.

3. Results and Discussion

The typical microstructure at a fracture surface of the shells is revealed in the low-magnification SEM image of Figure 3. A plate-like structure can be attributed largely to aragonite crystals. The direction of the plates is perpendicular to the outer (upper part in the image) and the inner surfaces of the shell. The outer surface apparently has a less dense structure. Calcite is expected to be concentrated in the outer layer of the shell. The inner layer (lower part in the image) has apparently a denser structure. Usually, the inner part of the

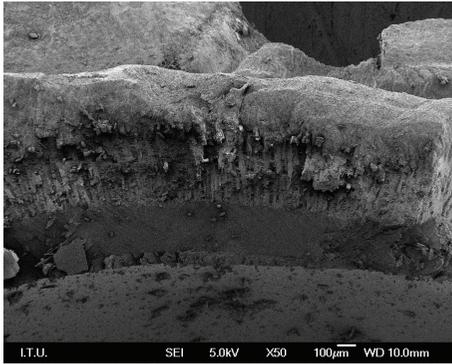


FIGURE 3: Microstructure at fracture surface of *Cerithium vulgatum* shell.

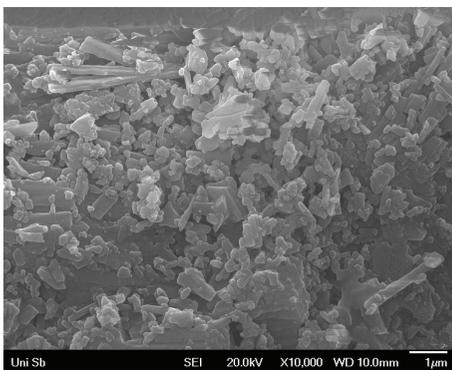


FIGURE 4: SEM image of raw powder after planetary milling and sieving with a sieve of $100\ \mu\text{m}$.

shells is largely made of aragonite. Therefore, the raw powder that was subjected afterwards to transformation into calcium phosphates should involve both the phases of aragonite and calcite, which is observed in all regular shells. But the influence of aragonite-calcite sea conditions on the evolution of biocalcification remained up to now poorly understood [34].

The nanopowders were spontaneously fabricated after crushing, milling, and sieving, as shown in the SEM image of Figure 4. The powder mainly consisted of prismatic particles with a semirounded shape and nanosize dimensions of about 200 nm. Some elongated rod-like prismatic particles with a length of ca $1\text{--}1.5\ \mu\text{m}$ and a width of ca 200 nm are also observed.

The results of the differential and gravimetric thermal analysis (DTA/TGA) of the raw powders after milling and sieving are plotted in the diagrams of Figure 5. The decomposition of CaCO_3 to CaO was clearly obtained in both curves. These curves confirm that the shell was exclusively consisted of CaCO_3 . Thus, the calculation of the amount of H_3PO_3 solution required to satisfy the demanded Ca/P ratios was possible.

The X-ray analysis of the powders produced after calcination for 4 h in air is shown in the diffractograms of Figure 6(a), for the powder B (Ca/P = 1.5, 400°C), and Figure 6(b), for the powder A (Ca/P = 1.667, 800°C). From

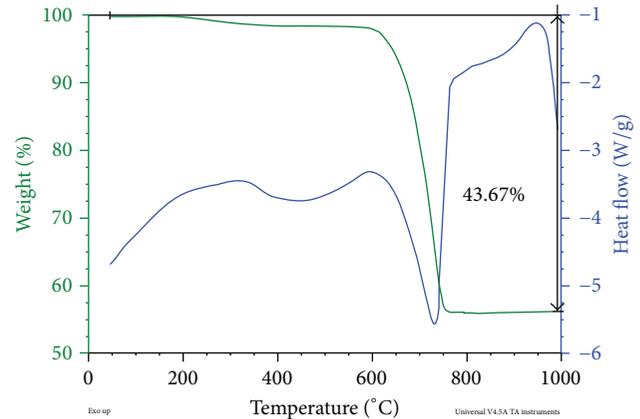


FIGURE 5: Differential and gravimetric thermal analysis (DTA/TGA) of the raw powders (after planetary milling and sieving).

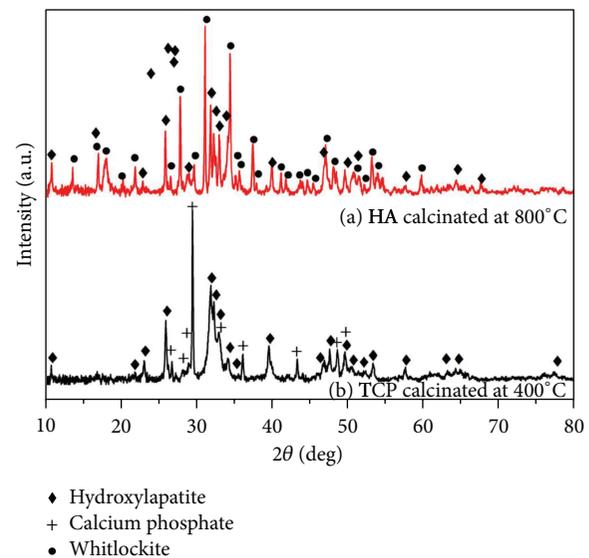


FIGURE 6: X-ray diffractograms of the produced powders after calcination for 4 h in air: (a) powder B (Ca/P = 1.5, 400°C); (b) powder A (Ca/P = 1.667, 800°C).

these diffractograms it is concluded that the transformation of CaCO_3 , in the form of either aragonite or calcite, was completely indicated. In both diffractograms, the phase of HA was clearly observed (JCPDS card 00-009-0432 in sample B and JCPDS card 01-089-4405 in sample A; the differences between the two cards are negligible). The second major phase recorded was TCP ($3\text{CaO}\cdot\text{P}_2\text{O}_5$) in particular β -TCP in the sample B (JCPDS card 00-009-0346) and whitlockite (JCPDS card 00-009-0169) in the sample A. Whitlockite is also known as β -tricalcium phosphate (β -TCP) [35], which is used in treatment of defects of cortical and cancellous bone due to its osteoconductivity and bioresorbability [36].

The findings of the X-ray analysis indicate that the powders produced are biphasic materials, which comprise HA and TCP. It is well known that the best bioceramic materials

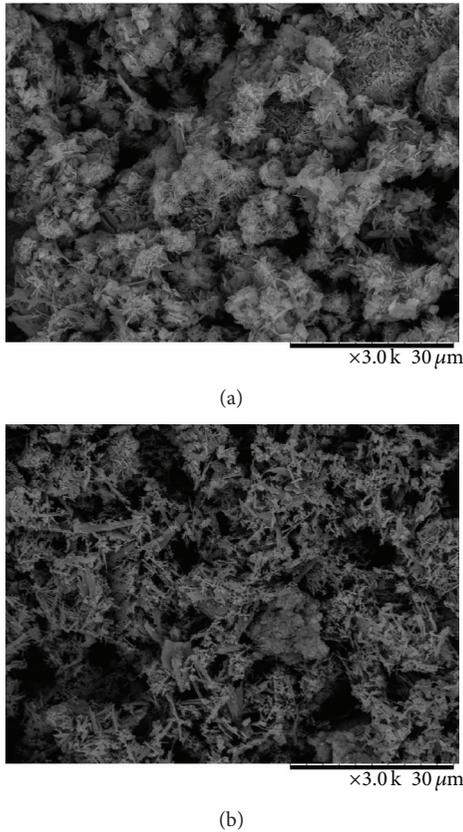


FIGURE 7: Microstructure of the produced powders after calcination for 4 h in air: (a) powder B (Ca/P = 1.5, 400°C); (b) powder A (Ca/P = 1.667, 800°C).

should ideally consist of biphasic materials of HA and β -TCP. In such biphasic biomaterials, β -TCP is the resorbable and osteoconductive [37] component. Usually, resorbable bioceramics are considered as very active and thus they stimulate a faster formation of the newly formed bone. On the other hand, HA presents an excellent biocompatibility and bioactivity.

All the results indicate that the produced powders are very promising materials. However, these good prospects, due to the biphasic crystalline regime of the produced powders, are further enforced because the SEM analysis showed that this production process resulted in the production of nanopowders as well. The characteristic microstructure of the powders produced after calcination for 4 h in air is shown in the SEM images of Figure 7(a), for the powder B (Ca/P = 1.5, 400°C), and Figure 7(b), for the powder A (Ca/P = 1.667, 800°C). The powder B comprises prismatic nanoparticles as well as needle-like nanorods with length of 1.5–3 μm and diameter of 200 nm. The SEM image of Figure 7(b) for the sample A indicates that this powder is apparently finer than the powder B because there are less prismatic particles; the rod-like (needle-shaped) particles are thinner (with a diameter of ca 150 nm), and formation of apparently loosened agglomerations of nanoparticles was also observed, as revealed in the middle of Figure 7(b).

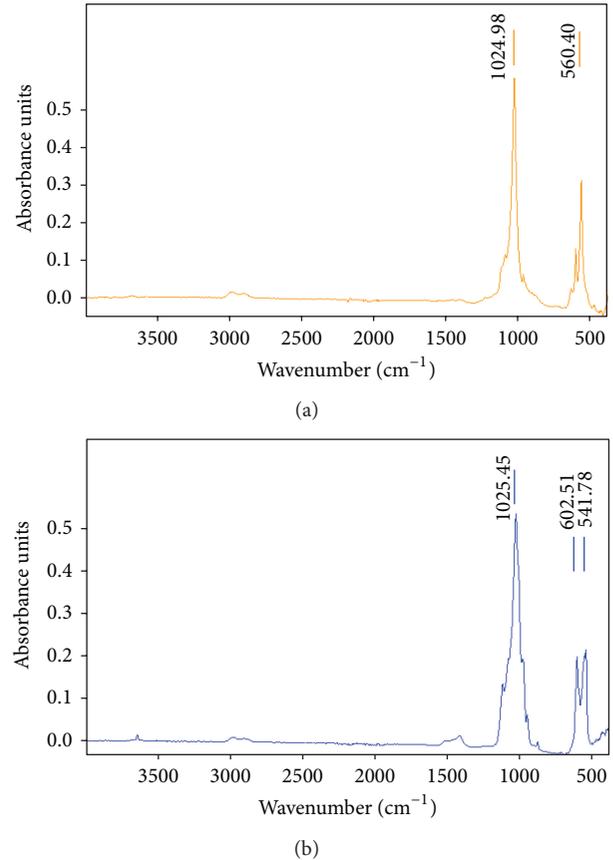


FIGURE 8: FT-IR spectra of the produced powders after calcination for 4 h in air: (a) powder B (Ca/P = 1.5, 400°C); (b) powder A (Ca/P = 1.667, 800°C).

FT-IR spectra of the HA powders in the range 4000–400 cm^{-1} are revealed in Figure 8 for powder B (Ca/P = 1.5, 400°C, Figure 8(a)) and powder A (Ca/P = 1.667, 800°C, Figure 8(b)), after calcination for 4 h in air. They revealed strong vibrations modes at the following wave numbers: 541, 560, 602, 1024, and 1025 cm^{-1} . Absorption bands characteristic of O–P–O bending vibrations can be clearly seen at 541 and 602 cm^{-1} . The powders appear to lack the O–H vibrational bands indicated by the weak peak at 630 cm^{-1} (Figure 8(a)) [38]. The sharp bands at 1024–1025 cm^{-1} correspond to ν_3 asymmetric stretching modes of $(\text{PO}_4)^{3-}$ groups. Moreover, the increase of the calcination temperature to 800°C caused the appearance of the peaks at 1500 and 3700–3500 cm^{-1} . The addition, IR bands in the range of 3700–3500 cm^{-1} (Figure 8(b)) were also observed by Duta et al. [39], which was assigned them to the O–H stretching vibrations of surface P–OH groups. These spectra are in agreement with the XRD results.

4. Conclusions

Using a simple mechanochemical method, biphasic bio-ceramic nanopowders of hydroxyapatite (HA) and tricalcium phosphate (TCP) were fabricated. It has been indicated that

these nanopowders can be used as bioceramic for graft material. The shell of a regular sea snail from the species *Cerithium vulgatum* was successfully transformed into the target compounds. This new method is very simple, economic, and safe.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

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